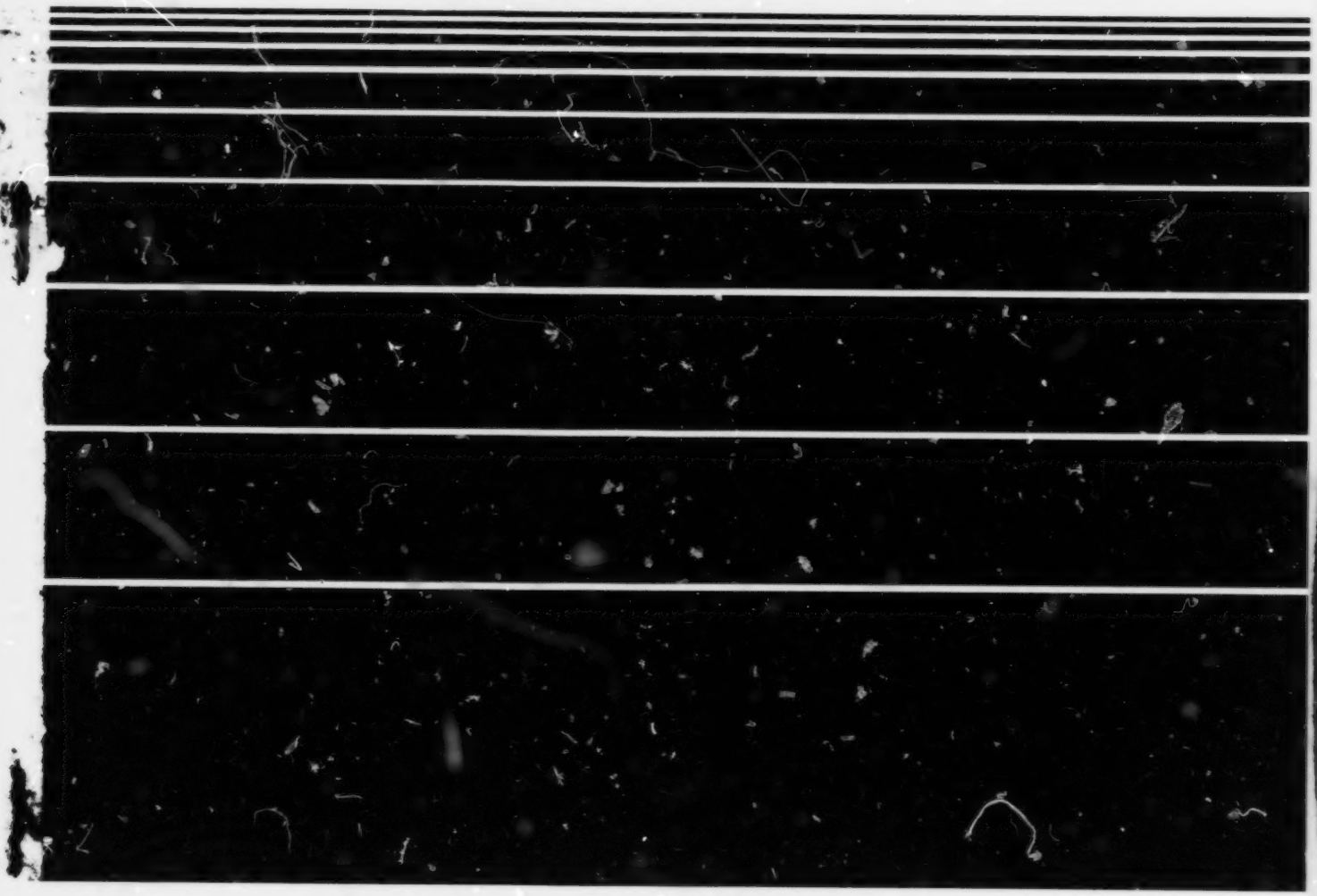

Manitoba
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and Workplace
Safety and Health

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GLYPHOSATE RESIDUES IN VEGETATION AND SOILS OF MANFOR SITES AFTER ROUNDUP APPLICATION IN AUGUST, 1985

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ABSTRACT

In August 1985 Manfor applied Roundup herbicide aerially (helicopter) to three forest blocks in the area of The Pas, Manitoba. The objective was to test its effectiveness in controlling hardwood competition in softwood plantations and also to test its effectiveness as a site preparation tool. Application rates of 3, 4 and 5 L/ha were tested.

Terrestrial Standards and Studies of the Department of Environment and Workplace Safety and Health, Province of Manitoba, conducted monitoring to determine the distribution, fate and persistence of the active ingredient, glyphosate, in the environment.

The amount of glyphosate intercepted by mylar plates was used to estimate actual spray deposition. Same day, 32 day and 295 day post-spray samples of aspen foliage and soil were analyzed for glyphosate residues.

The 3 L/ha rate was not as effective in killing vegetation as the 4 or 5 L/ha rate. The mylar deposition monitors provided good estimates of actual spray distribution. Initial concentrations of glyphosate in aspen leaves should not be toxic to birds or animals which might ingest them. Glyphosate residues deposited on the soil were adsorbed entirely by the surface organic material and no downward leaching occurred. Rapid breakdown of soil residues occurred. The half-life of glyphosate in forest soils of The Pas area appears to be less than 16 days.

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1. INTRODUCTION

Roundup, a non-specific herbicide, is being used as a forest silvicultural tool to reduce hardwood, shrub and herb competition in conifer plantations. Roundup has full registration for forestry use in Canada and is often applied by aerial spraying. The active ingredient in Roundup is glyphosate (N-(phosphonomethyl)glycine) which kills vegetation by inhibiting ESP-synthetase, an enzyme which catalyzes the formation of 3 amino acids (Jaworski 1972). The herbicide enters the plant through the leaves and is translocated to meristematic tissues especially those in the roots.

In August 1985, Manfor sprayed Roundup on three blocks with the permission of the Forest Management Advisory Committee, on the condition that the Manitoba Department of Environment and Workplace Safety and Health conduct a monitoring program. Terrestrial Standards and Studies Section designed and carried out monitoring to determine the distribution, fate and persistence of glyphosate in the terrestrial environment. Manfor covered all operational and analytical costs of the monitoring program. The results are presented in this report.

2. METHODS

Roundup was sprayed by helicopter on the trial spray blocks on August 22 and 23, 1985. Blocks 1 and 2 were along the Atik Lake Road, 13 km and 6 km west of Highway 10 respectively (Figure 1). Moose Lake block M-16 was along the Crossing Bay Road, 125 km northeast of The Pas.

Blocks 1 and 2 were sprayed with Roundup at 4 L/ha and 3 L/ha respectively to test its efficacy as a conifer release tool. Both blocks had been planted in 1982, Atik L. 13 km to black spruce container stock and Atik L. 6 km to 3 year old white spruce container stock. Moose Lake block M-16 was sprayed with Roundup at 5 L/ha to test its efficacy as a site preparation tool.

Monitoring of actual spray deposition and glyphosate residues in vegetation and soils was carried out.

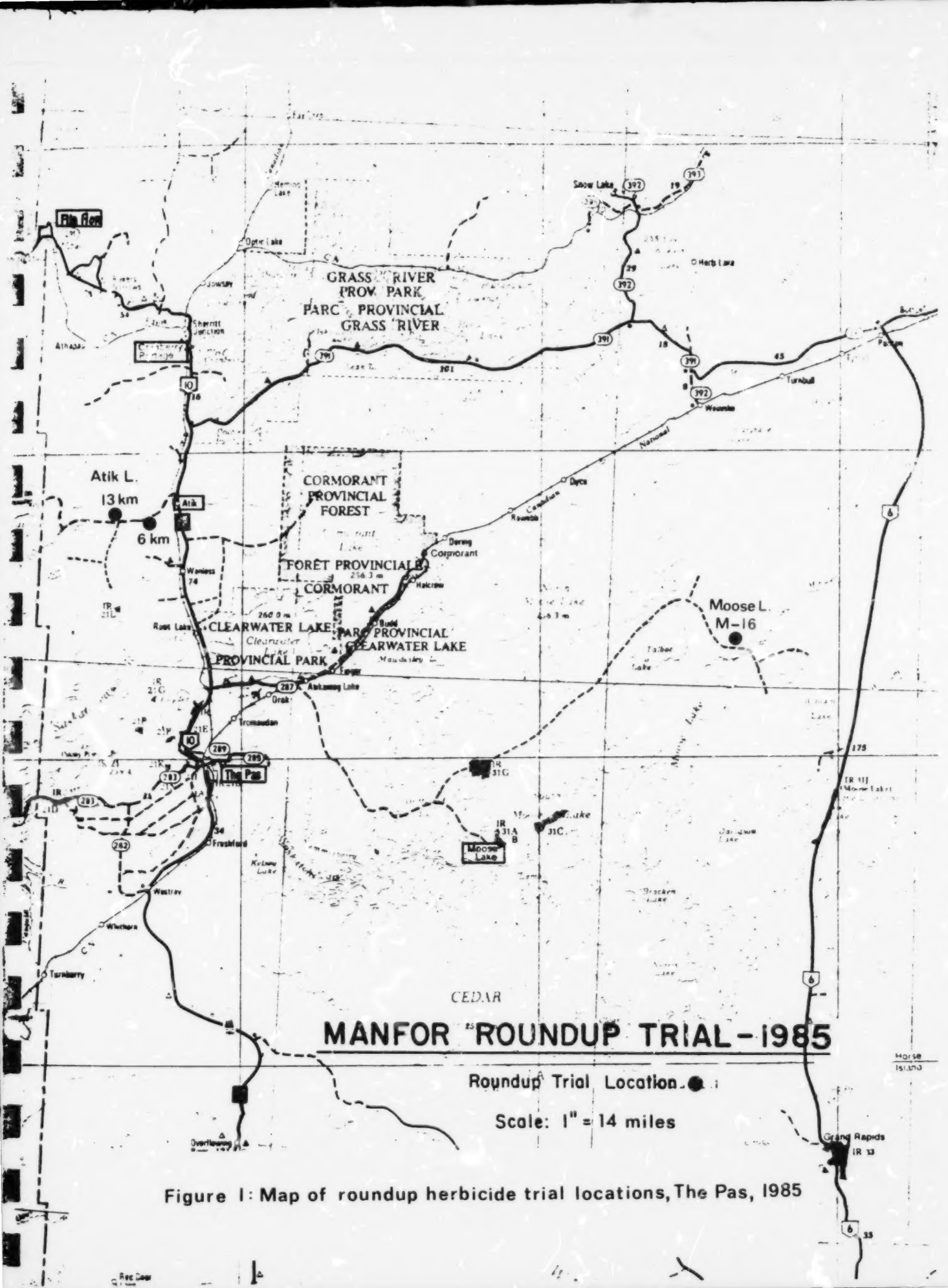

Figure 1: Map of roundup herbicide trial locations, The Pas, 1985

2.1 Deposition Monitoring

Deposition monitoring was conducted to provide an estimate of the actual amount of spray which reached the ground in the immediate vicinity of the vegetation and soil sampling sites. Four sites were monitored in each spray block (Figures 2, 3 and 4) and where feasible, the monitors were located close to the efficacy plots established by Manfor. Three monitors were placed within the spray area of each block and the fourth (control) was located in the peripheral unsprayed zone of each block.

Each deposition monitor consisted of a wooden platform 31 cm x 31 cm nailed to the top of a 1.2 m post (2 x 4) which had been sharpened and driven into the ground.

Two 6 in. x 6 in. (464.5 cm^2) squares of mylar were attached to each platform with thumbtacks. The platform was covered with aluminum foil to provide a clean backing for the mylar monitors.

The mylar sheets were set out just before each block was sprayed and were collected immediately after the spraying was completed. The two sheets and thumbtacks from each monitor were placed in a clean plastic container with a tight fitting lid (500 g honey container). They were immediately placed in a cooler on dry ice and shipped to Winnipeg where they were stored in a freezer at -18°C.

2.2 Vegetation Sampling

A pre-spray and an immediate post-spray sample of aspen foliage was collected from each site in each block. The area around each deposition monitor was divided into 4 quadrants, northeast, southeast, southwest and northwest (Figure 5). The pre-spray sample was collected from the aspen sapling nearest to the monitor in the northeast quadrant. The immediate post-spray sample was taken from the closest aspen sapling in the southeast quadrant.

The leaves were stripped (pulled off by hand) from all branches along one side of the sapling. Disposable rubber gloves were worn when collecting post-spray samples to prevent skin contact with the herbicide. Gloves were changed between each sample to prevent cross contamination.

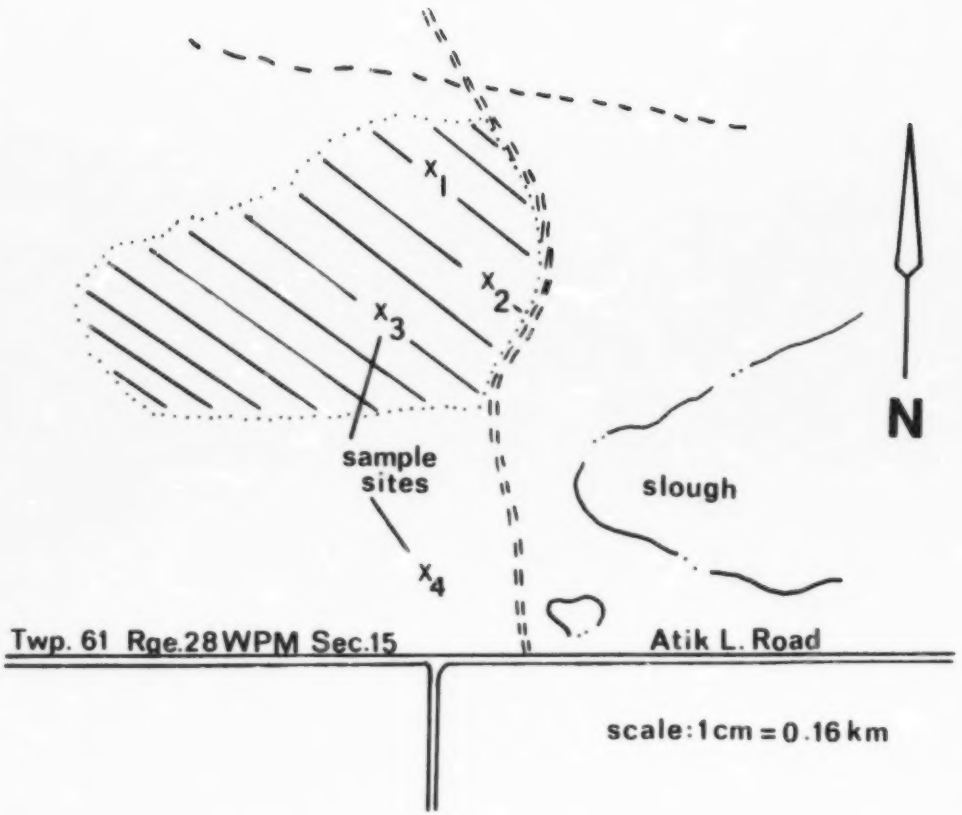

Figure 2: Sampling design at Atik Lake – 13 km spray block

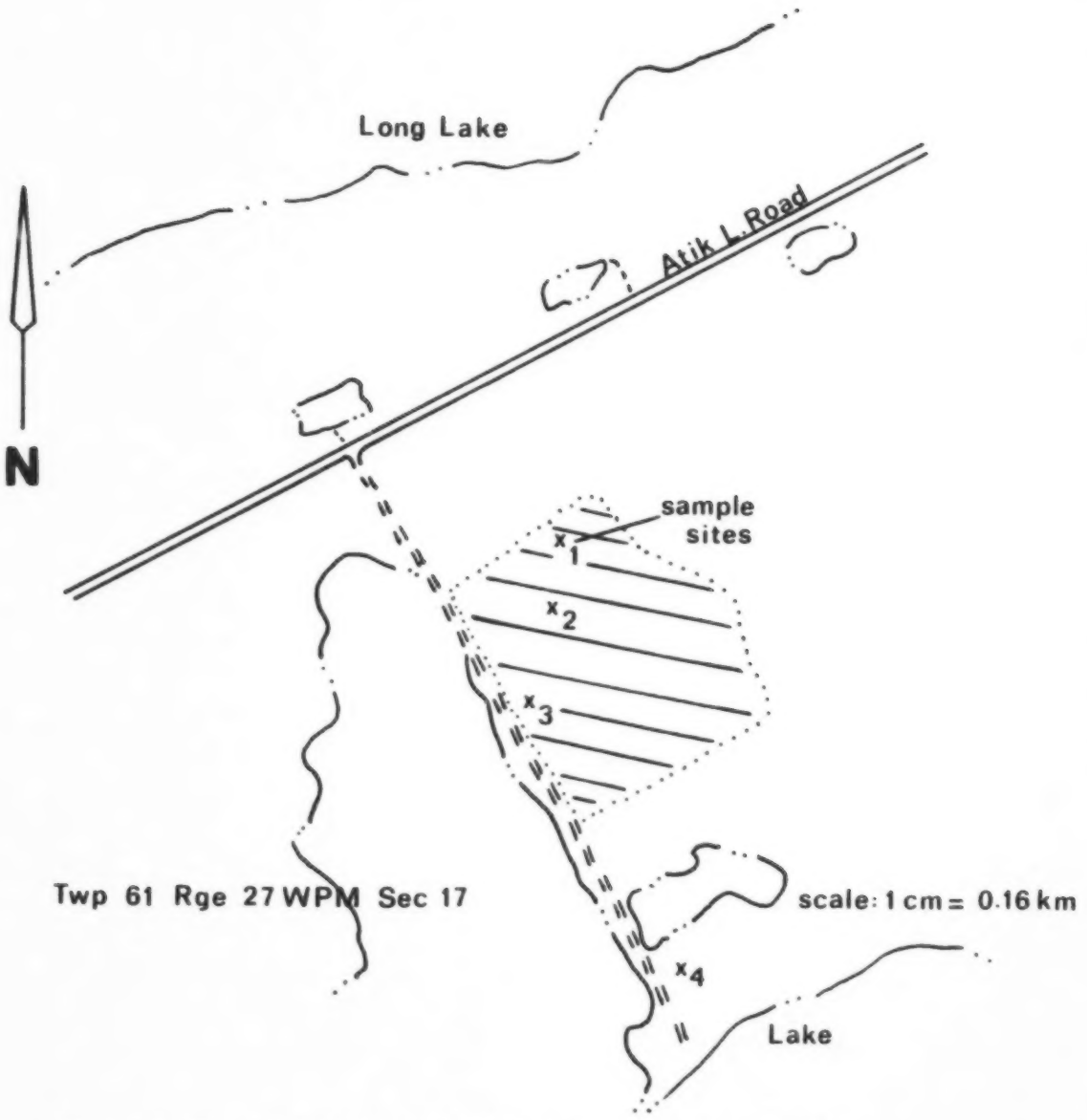

Figure 3: Sampling design at Atik Lake – 6 km spray block

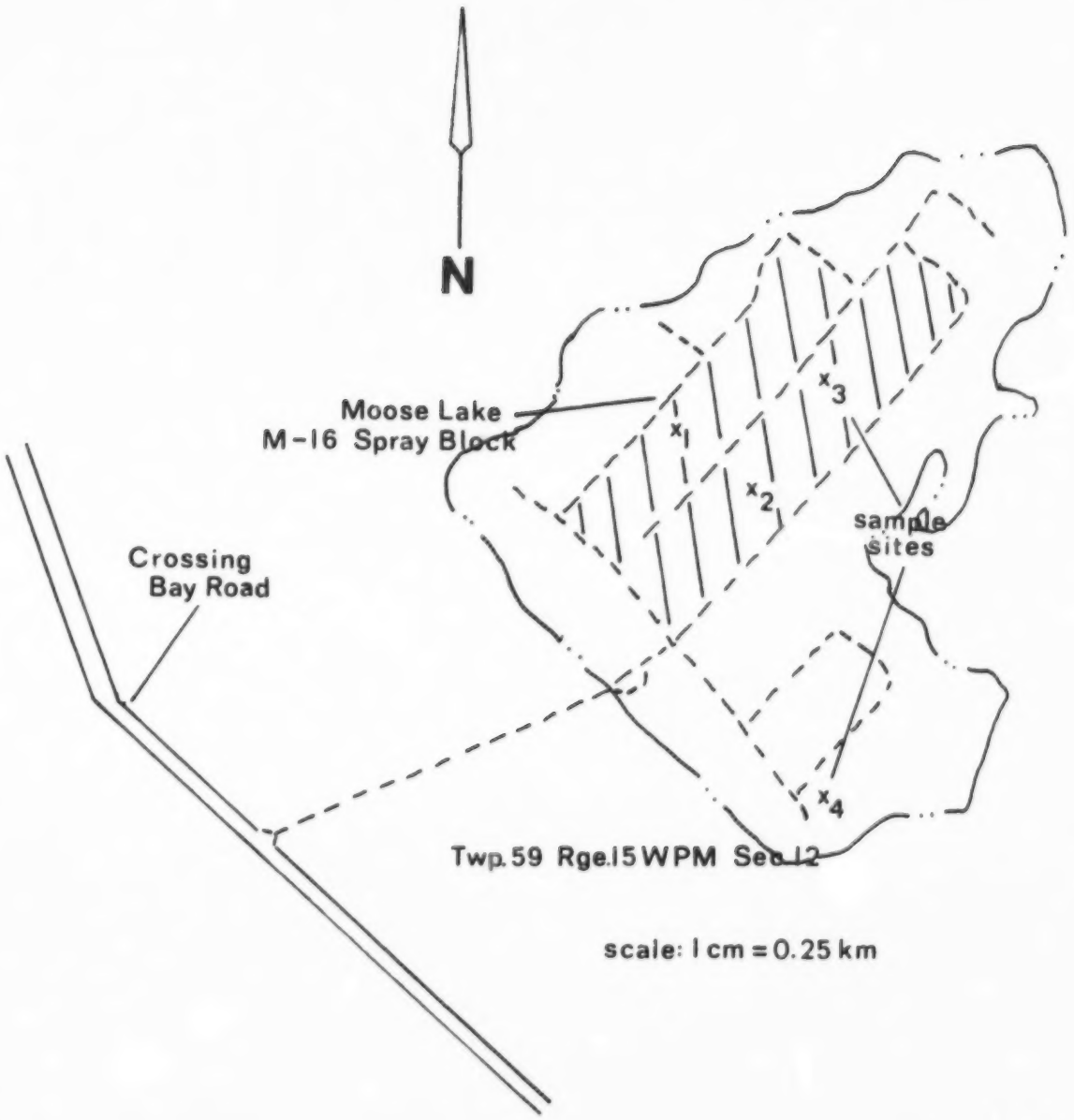

Figure 4: Sampling design at Moose Lake M-16 spray block

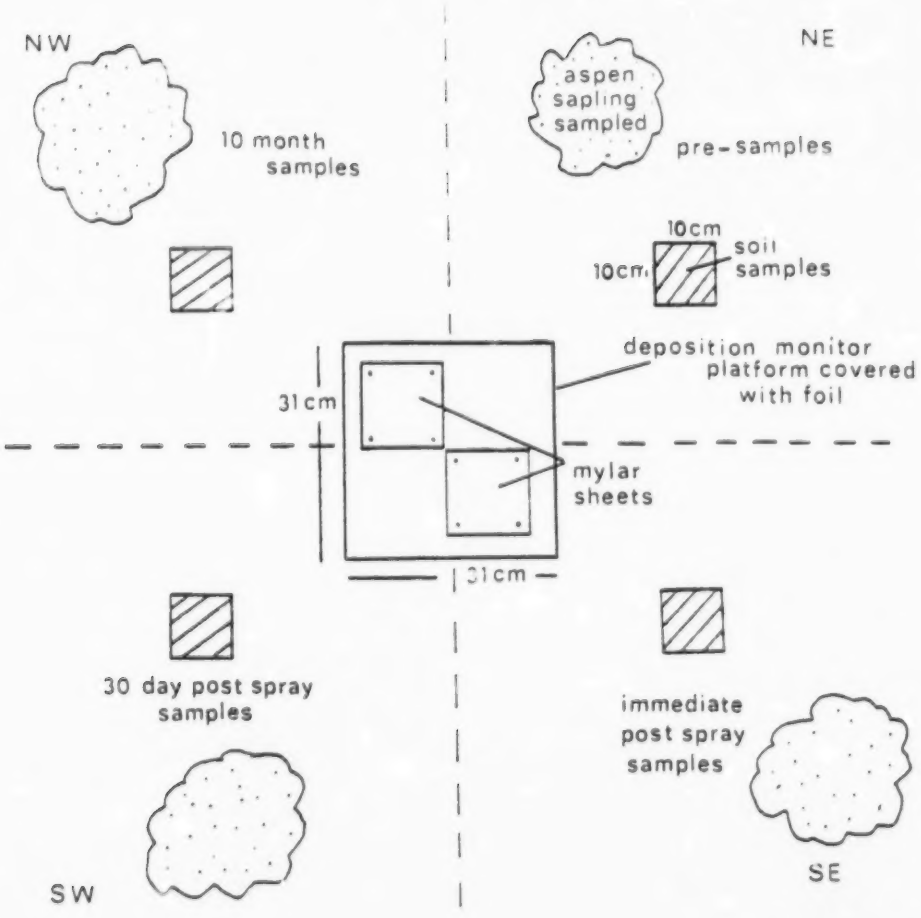

Figure 5. Sampling design at each residue monitoring site

The leaves were placed in clean plastic containers with tight fitting lids (500 g honey containers) and placed on dry ice in a cooler. The frozen samples were transported to Winnipeg and stored in a freezer at -18°C.

2.3 Soil Sampling

Soil sampling locations were selected within each of the four quadrants used for vegetation sampling (Figure 5). One quadrant at each site was sampled at each time e.g. pre-spray-NE, immediate post-spray-SE, 32 day post-spray-SW and 295 day post-spray-NW. The other selection criteria were that the sample location be close to but not shielded by the deposition monitor and in an area which was not protected by overstory vegetation.

A standardized 10 cm x 10 cm soil area was sampled from three depths - the surface organic layer plus the 10 cm and 30 cm depths (Figure 6). We placed a 10 cm x 10 cm template on the soil surface and cut around it with a sharp knife. The block of organic material was carefully lifted out and placed in a pre-washed plastic container. A soil pit was dug alongside the sampling location to a depth of 40 cm. The wall of the pit next to where the organic sample had been removed was trimmed vertical with a clean trowel. Soil from beneath the organic sample was removed to the 10 cm depth and discarded. A clean putty trowel was used to remove a 10 cm x 10 cm x 2 cm thick slice of soil. The sample was placed in a clean plastic container. Soil was again removed and discarded down to the 30 cm depth and the third sample, a 10 cm x 10 cm x 2 cm thick slice was removed and placed in a plastic sample container. All soil samples were frozen by placing them in a cooler with dry ice. They remained on dry ice during transit to Winnipeg where they were stored in a freezer at -18°C.

2.4 Laboratory Analysis

Samples were analyzed for glyphosate residues by Wastex Industries Inc. Pottstown, Pennsylvania, U.S.A. The samples were shipped on dry ice by overnight courier. Deposition samples were

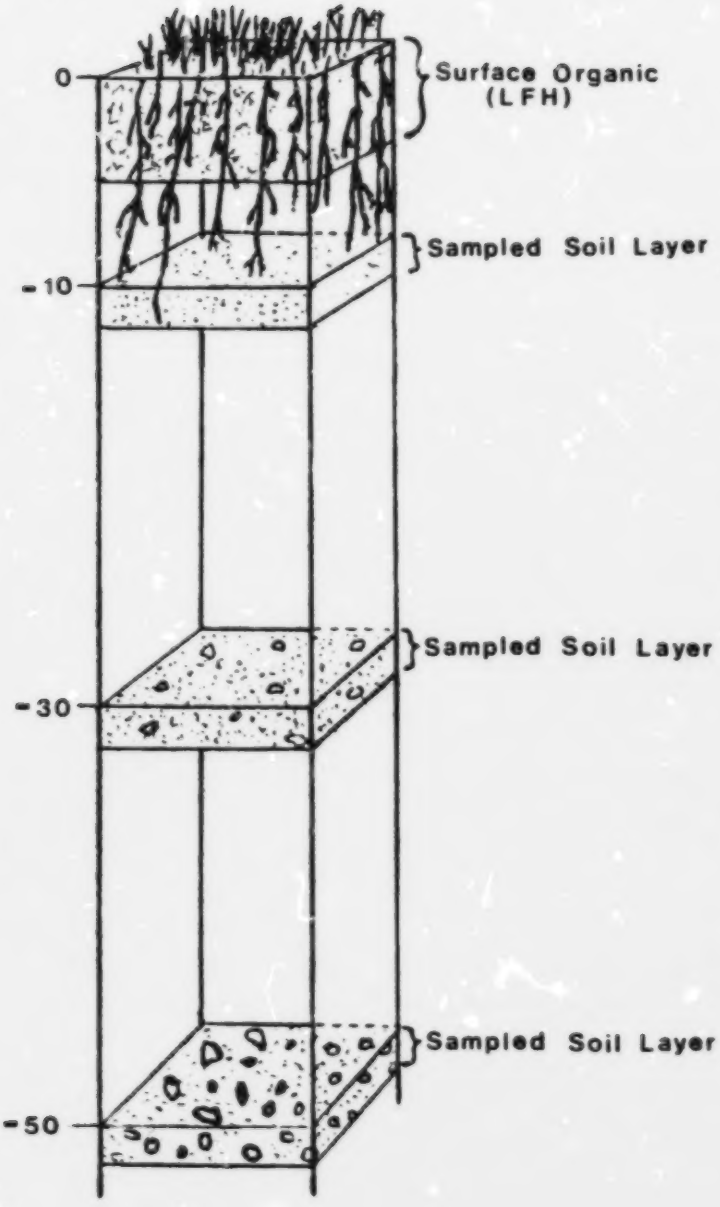

Figure 6: Diagram showing soil profile sampling

analyzed by rinsing the mylar sheets, thumbtacks and plastic containers with 1N HCl and analyzing for total glyphosate. Vegetation and soil were air dried on arrival at the laboratory. When dry, the samples were homogenized in a blender, extracted with a 1:3 solution of chloroform and 0.1N HCl, and analyzed by the HPLC - Fluorometric Method developed by Monsanto (confidential).

2.5 Data Analysis

All deposition data were reported by the laboratory as µg of glyphosate per plate. These were converted to standard deposition units using the following formula:

$$D = \frac{\mu g/plate}{A} \times 100$$

where D = deposition (g/ha)

A = area of mylar (464.5 cm^2) on each deposition monitor

100 = constant to convert from $\mu g/cm^2$ to g/ha

The soil residue data were reported as µg/g. These data were standardized to deposition units using the formula:

D = conc. x wt.

where D = deposition g/ha

Conc = concentration of glyphosate in soil µg/g

wt = dry weight of soil sample.

Because each soil sample had a surface area of 100 cm^2, the product of conc x wt calculates the total weight (µg) of glyphosate in a 100 cm^2 area or µg/100 cm^2 which is equivalent to g/ha.

3. RESULTS AND DISCUSSION

3.1 Spray Conditions

Weather conditions were recorded on site by Manfor before the spraying started and again after the spraying was finished (Table 1). Other observations which are of interest were made by Terrestrial Standards and Studies personnel before, during and after the sampling.

Table 1: Weather conditions during aerial application of Roundup on Manfor blocks in August, 1985.

Block	Date	Time	Cloud Cover %	Temp. °C	Relative Humidity %	Wind	
						Dir.	Speed (km/h)
Atik (6 km)	22/8/85	1834	95%	17	95%	SE	4
Atik (13 Km)	22/8/85	1930	95%	17	95%	SE	4
		2005	90%	16.5	95%	SE	4
Moose L. M-16	23/8/85	1015	25%	16.5	90%	NW	4
		1053	25%	18	82%	NW	5

Rain fell the morning of August 22 but stopped shortly after noon. The foliage was still wet when both Atik Lake blocks were sprayed. When Atik L. 6 km was sprayed, the helicopter pilot noted lateral spray drift of approximately 2 swath widths. That suggested that the wind 70 feet above ground was stronger than at ground level. The pilot took this drift into account when completing the block, to prevent drift onto the control area along the north side.

It is likely that this "upper wind" diminished by the time the second block (Atik L. 13 km) was sprayed later in the evening. During sampling, we observed very calm conditions which allowed fog to form within one hour after the spraying.

Because of the recent rainfall and high humidity conditions, the aspen foliage was too wet for proper sampling; water droplets on the surface of the leaves, which probably contained herbicide, were lost.

At the Moose Lake M-16 block, the fog lifted at about 7:00 a.m., August 23, 1985, although there was still patchy fog in the area. The wind was light but during the spray operation, a few gusts up to 10 kmph were registered on the anemometer. The sky was clear and the air temperature was rising; this condition likely produced thermals which could have affected spray distribution. All of the dew had evaporated from the foliage by the time the vegetation sampling was started.

3.2 Roundup Deposition

The deposition to the monitors (Table 2) was quite variable and was generally lower than expected (calculated from application rates and area of the mylar).

Surrogate monitors such as these mylar sheets may underestimate the actual deposition to natural receptors such as leaves. The larger size and flat horizontal configuration may produce unnatural air currents around the monitor which could deflect droplets away from the surface. There was no feasible way of testing the efficiency of the monitors but they seemed to perform well because the results were often similar to those expected and correlated well with foliage residues (r^2 = 0.93 for wet foliage and r^2 = 0.99 for dry

Table 2: Glyphosate deposition and residues in foliage and soil after aerial application of Roundup to forestry plots in The Pas area.

Block* -Site	Applic Rate	Deposition g/ha		Foliage µg/g			Surface Organic Soil g/ha			
		Expected	Actual	Pre	Immed Post	295 day	Pre	Immed Post	32 day	295 day
1 - 1	4L/ha	1424	1566	<0.05	36	N/A	<3.0	171.0	11.6	<2.2
1 - 2	4L/ha	1424	1630	<0.05	30	0.24	<3.0	<3.2	14.9	2.5
1 - 3	4L/ha	1424	1029	<0.05	20	N/A	<3.0	103.3	9.4	2.4
1 - 4	0	0	0.22	<0.05	<0.05		<3.0	7.0	4.4	4.4
2 - 1	3L/ha	1068	525	<0.05	13	0.44	<3.0	65.8	10.8	<3.5
2 - 2	3L/ha	1068	1005	<0.05	22	0.57	<3.0	163.6	15.2	<2.8
2 - 3	3L/ha	1068	658	<0.05	21	0.31	<3.0	45.4	9.3	<2.5
2 - 4	0	0	<0.05	<0.05	<0.05		<3.0	5.8	<2.0	<2.7
3 - 1	5L/ha	1780	945	<0.05	76	N/A	<3.0	432.2	6.9	<1.5
3 - 2	5L/ha	1780	690	<0.05	58	N/A	<3.0	70.7	7.2	<1.9
3 - 3	5L/ha	1780	868	<0.05	64	N/A	<3.0	61.0	7.6	9.2
3 - 4	0	0	8.2	<0.05	0.40		<3.0	<1.5	<1.2	<1.6

* 1 = Atik L. 13 km; 2 = Atik L. 6 km; 3 = Moose Lake M-16

N/A = Not Available; aspen killed

foliage). This is in contrast to the findings of Newton et al. (1984) who concluded that mylar sheets are poor monitors of deposition.

In some cases less than half of the expected amount of glyphosate was found on the monitors while at two locations (1-1 and 1-2), deposition was greater than expected. We can only speculate on how the latter might have occurred. Under normal spray conditions, the spray fans out to provide fairly uniform coverage of a swath that is wider than the spray boom. However, when wind speed is low and humidity is high, the spray droplets may increase in size as they coalesce with water vapor in the air during their fall. This would increase their rate of fall. If this occurred, the spray could be concentrated along the centre of the spray swath and could account for the higher than expected deposition.

A slight amount of spray deposition was found at site 1-4, the control. This should not have occurred because the control area was south of the treated area and the SE wind should have moved any drift in the opposite direction.

Deposition at site 2-1 was less than half the expected amount. There seemed to be sufficient wind for good distribution of the spray swath and we have no data to indicate why this variability occurred. Vagaries in the wind speed could have been a factor but there is also an inherent lack of precision in aerial application especially when the helicopter is flying at a height of 18 to 24 m (60 to 80 ft.).

Deposition at the Moose Lake M-16 block was only 39 to 53% of that expected and there was some drift toward the control site (0.5 km downwind of the treatment area). We suspect that weather conditions may have contributed to the drift. The wind speed was within the required range but by 10:15 a.m., the air temperature was increasing at a rate of 1.5°C per hour. The fall rate of small droplets could have been impeded by rising thermals which develop under these conditions. This could have increased the residence time of droplets in the air thus providing more opportunity for downwind drift.

3.3 Herbicide Effects

Observations were carried out in June of the following year (i.e. 1986) to determine the effect of the herbicide on vegetation at the three treatment areas. The Atik L. 6 km block had the lowest application rate (3 L/ha) and the proportion of vegetation which was killed was distinctly lower. Although many aspen saplings and shrubs failed to leaf out many others did leaf out but had obvious signs of herbicide injury (chlorotic, small leaves often with rolled edges). Many grasses and herbs were still alive but also obviously stressed, exhibiting poor colour and non-vigorous growth. One small portion of the block did not suffer any obvious damage and we conclude that it was inadvertently missed by the spray either because of pilot error or because of vagaries in wind drift.

The Atik L. 13 km block was sprayed with Roundup at 4 L/ha and with the exception of one swath width which appeared to have been missed, nearly 100% of the aspen, birch and shrubs were killed. Many of the grasses and herbs in the ground cover were also dead but in some places they were still alive although showing signs of stress (chlorotic and/or misshapen leaves). Coincidentally, one of the monitoring locations (1-2) was along the swath which was missed. Although glyphosate deposition was much higher than expected (1630 g/ha vs 1424 g/ha expected) and the concentration of glyphosate in the aspen foliage was 30 µg/g, there was no visible evidence of herbicide injury to the vegetation. There should have been almost total vegetation kill based on the significant damage at site 1-3 where deposition and foliage residues were lower. We do not have any plausible explanation for this anomaly.

At the Moose Lake site, nearly 100% of the vegetation was killed but one swath was missed there as well. Herbicide drift damaged approximately two swath widths along the east side of the Moose Lake spray block but there was no evidence of vegetation injury at the control site 0.5 km downwind.

Although a high proportion of the trees and shrubs in the two Atik L. spray blocks were killed, many herbs and grasses were shielded by the overstory vegetation and survived. Thus although there was

considerable habitat loss, some still remained. At the Moose Lake spray block the saplings and shrubs were smaller and more scattered. With less overstory cover, more of the spray impacted the herbaceous layer. In spite of that, a few plants were shielded by scattered logs or by the more vigorous saplings and shrubs so there wasn't total vegetation kill. However, it is important to note that Roundup is not a soil sterilant and grasses and herbs will revegetate the treated areas within a year or two, thus providing an early successional type of habitat. It is also important to remember that the buffer strips along the periphery of the spray blocks are not sprayed and will remain for wildlife use.

3.4 Foliage Residues

Examination of the foliage residue columns in Table 2 reveals that no glyphosate residues were found in the pre-spray samples of aspen leaves.

Post-spray glyphosate residues on aspen foliage were proportionally lower at the Atik Lake blocks than at Moose Lake. For example, the glyphosate concentration in aspen foliage at Moose Lake was twice as high as at Atik L. 13 km, even though the application rate was only 25% higher. We believe that this was caused by the conditions at the time of sampling; when the immediate post-spray samples were collected at the Atik Lake blocks, the leaves were wet with residual rain/dew/fog. It is likely that the spray was dissolved in those water droplets. Since much of the surface water dripped off when the leaves were being picked, a substantial portion of the glyphosate residue may have been lost as well. By comparison, the foliage at Moose Lake was dry and no loss would have occurred during sampling.

The glyphosate residues in the foliage at Moose Lake M-16 were lower than those reported by Newton *et al*. (1984). They reported mid-crown concentrations of 257 $\pm$ 161 µg/g after an application of 9.5 L/ha. Based on these data and assuming a linear relationship between application rate and foliage residues, a mean concentration of 135 µg/g could have been expected at Moose Lake. Instead, the mean

concentration was 66 ± 9.2 µg/g. This was consistent with the results of the deposition monitoring; only 50% of the expected amount of glyphosate was deposited. This may have been partly due to off-site drift losses. The foliage residue data from the downwind control site confirms that there was drift; aspen leaves at site 3-4 had a glyphosate concentration of 0.40 µg/g. That amount apparently did not constitute a toxic dose, because no visible injury was observed in June 1986.

The immediate post-spray residues in aspen foliage are well below those which would be toxic to birds and wildlife which might eat it. Acute toxicity in birds such as quail only occurs when high concentrations of glyphosate are ingested (LC_{50} >4600 µg/g) (Serdy, F. 1980. cited in Ghassemi et al 1982). Quail are close relatives of roughed grouse which are present in the forests around The Pas. Long-term feeding studies on rodents such as rats and mice indicate no adverse effects when up to 300 µg/g glyphosate were added to the daily food supply (Monsanto Canada Inc.). Similarly, carnivores (test species beagle dog) were not affected by long-term feeding with 300 µg/g in the food.

In June 1986, aspen foliage from the Atik L. 6 km block had small but detectable residues ranging from 0.31 to 0.57 µg/g. The saplings appeared to have sustained near fatal herbicide damage, because the leaves were small and chlorotic and had rolled margins. At site 1-2 within the missed swath at Atik L. 13 km, there was a slight trace of glyphosate (0.014 µg/g) in the aspen leaves. There was no visible damage to the vegetation in that area. These trace concentrations in the new growth indicate that not all of the herbicide which enters the plant through the leaves, becomes bound in the stem and root tissues. The "free" glyphosate can be translocated back into the new growth.

3.5 Soil Residues

Residues of glyphosate were only found in the surface organic layer of soil. Data for the 10 cm and 30 cm depths are not presented in Table 2 because no residues were detected in those mineral subsoil

samples. This is consistent with previously published data indicating that glyphosate binds readily to organic material (Sprankle et al. 1975).

Soil sampling sites were chosen in an area with no vegetation cover, to try to standardize the exposure of each replicate to the spray. Random sampling would have created the potential for one replicate to be located in an exposed area and another under vegetation. Data would not have been comparable from one replicate to the other because different amounts of glyphosate would have been intercepted by the vegetation. Standardizing the soil sampling to open areas did mean that the glyphosate residues which were found represent the maximum in the range. Other soils in the spray block which were covered by vegetation would have had lower glyphosate residues. Consequently, the overall average soil residues were probably much lower than indicated by these data.

In spite of the efforts to standardize the exposure of the soil replicates, the glyphosate residues were still variable. Differences in the size, shape and position of nearby trees, saplings and shrubs probably affected the micro-air currents within these small openings in the vegetation cover. This could have varied the amount of spray deposited to the various replicate locations.

Immediate post-spray amounts of glyphosate in the soil were much lower than those recorded by the deposition monitors. At most monitoring locations, the glyphosate in the soil was only 10% of the actual deposition. Sites 2-2 and 3-1 were exceptions, having 16% and 45% of the deposition amount respectively in the surface soil sample.

Two of the three control sites (1-4 and 2-4) had traces of glyphosate in the soil. The residue at site 1-4 parallels the residue on the deposition monitor which suggests that spray drift may have occurred. Drift should not have occurred because site 1-4 was upwind of the spray block. However, the light winds and high humidity may have produced unexpected movement in that direction. On the otherhand, the residue of 5.8 g/ha at site 2-4 appears to be an anomaly because there was no residue on the deposition monitor or on the foliage sample. Our only explanation is that an amino acid with

an analytical peak which could not be distinguished from glyphosate, may have been present in the soil sample (J. Cowell, personal communication, Monsanto Inc. Laboratory, St. Louis, Missouri). The control at the Moose Lake site (3-4) should have had detectable residues in the soil because it was downwind of the spray block, and the deposition monitor as well as the foliage analysis detected drift. However, because no glyphosate was found, we assume that not enough of the drift contacted the soil to accumulate a detectable residue.

Newton et al (1984) also found that glyphosate residues in the soil varied widely, ranging from 0.12 to 3.05 µg/g. By comparison, concentrations in treated areas in our study were higher (1.1 and 9.1 µg/g) even though the application rate was lower than that used by Newton et al. (1984) (9.25 L/ha). Dense overstory vegetation intercepted more of the spray in Newton's study.

Mean glyphosate residues in the surface organic layer decreased substantially over the initial 32 day post-spray period (Figure 7). Site 1-2 was an exception; glyphosate increased from a value below the detection limit to 14.9 g/ha (Table 2). The value of 14.9 g/ha is consistent with the deposition and immediate post-spray foliage residue data but again begs the question of why vegetation along the swath was not killed. After 32 days, a small residue still remained in the surface soil at site 1-4 providing further support that some drift must have reached that control site.

At most sites, the residue remaining after 32 days was about 10% of that present immediately after the spray. The highest percentage remaining was 20% at site 2-3. This indicates that the half-life of glyphosate was less than 16 days because with a half-life of 16 days, after 32 days, 25% would have been left (50% broken down in the first 16 days and 50% of the remainder or 25% in the next 16 day period). Ten to 12% remaining after 32 days indicates a half-life of 11 days or less. This is considerably less than the 29.2 day half-life reported by Newton et al. (1984) for forest soils in Oregon.

Further breakdown of the glyphosate in the soil occurred over the period from September 23/24, 1985 to June 17 and 18, 1986.

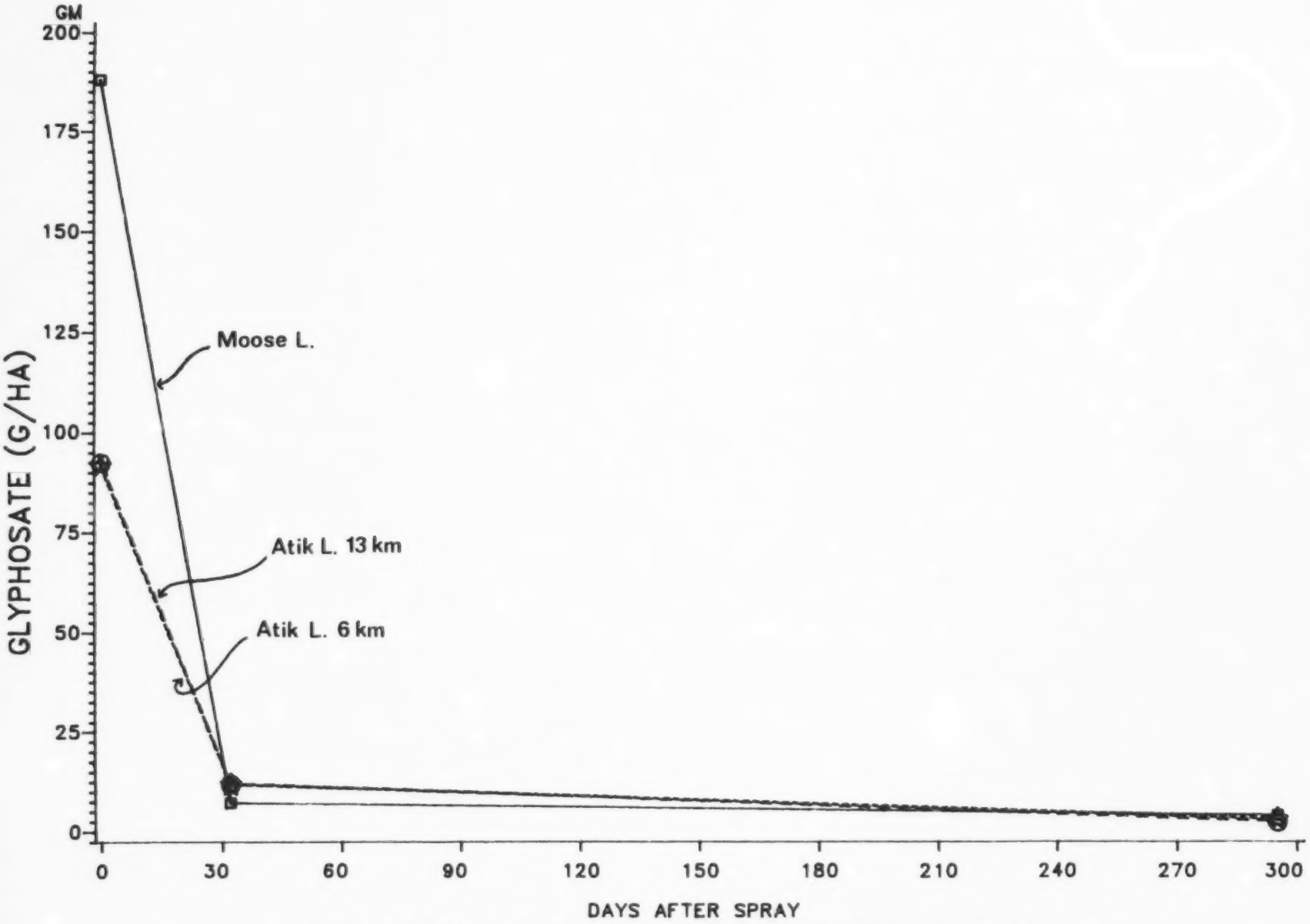

FIG.7 : MEAN GLYPHOSATE RESIDUES IN SOILS OF MANFOR SPRAY BLOCKS AUG.1985 TO JUNE 1986.

Residues were below detection limits at all but 4 sites, 1-2, 1-3, 1-4 and 3-3. Site 3-3 showed a slight increase from the 32 day sample but because of variability between microsites, this location may have had a higher concentration to begin with. The residues at the other sites were very small and close to the detection limit. We are still not sure why a residue was still present at the control site at the Atik L. 13 km block but it may have been an analytical anomaly caused by the presence of an amino acid.

It is important to note that the glyphosate which reached the soil was completely bound to the organic material at the soil surface. Breakdown occurred in the organic layer and no downward leaching occurred. The data also show that rapid breakdown occurs in the forest soils of The Pas area of Manitoba.

4. CONCLUSIONS

The 3 L/ha Roundup application rate was not as effective in killing vegetation as the 4 L/ha and 5 L/ha rate. However, even at the 5 L/ha rate some herbs and grasses survived in sheltered locations.

Weather conditions such as those which prevailed during the spraying of the Moose Lake site caused downwind drift and should be avoided in the future.

Initial concentrations of glyphosate in the aspen foliage were much lower than those reported in the literature. Saplings which were not killed by the spray had a small residue (≤3% of initial) in the new foliage the following year.

Based on acute and chronic toxicity data even initial concentrations in the foliage would not be expected to harm birds or animals.

Glyphosate deposition to the soil was variable and much lower than that found on the deposition monitors, even though replicate sampling locations were selected for maximum exposure to the herbicide. The herbicide which contacted the soil was totally adsorbed to the surface organic material and downward leaching did not occur.

Rapid breakdown of soil residues occurred during the initial 32 day period indicating a half-life of less than 16 days. Further breakdown occurred over the winter; in June 1986, slight residues remained in only 3 of 9 replicates from the treatment areas.

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